1.0 Title

Standard Operating Procedure for Determination of Phenolics, Total Recoverable in Water and Solid

2.0 Scope and Application

2.1 This method is applicable to the analysis of drinking, surface and saline waters and domestic and industrial wastes.

2.2 When the colored end product is extracted and concentrated in a solvent phase using phenol as a standard, this method is capable of measuring phenolic materials at the 5 µg/l level.

2.3 This method is capable of measuring phenolic materials at the 30 ug/l level in the aqueous phase (without solvent extraction) using phenol as a standard.

2.4 It is not possible to differentiate between different kinds of phenols using this method.

3.0 Summary of Method

3.1 This is a colorimetric method that determines phenol and ortho- and meta-substituted phenols. Phenolic materials react with 4-aminoantipyrine in the presence of potassium ferricyanide at a pH of 10.0 to form a stable reddish brown colored antipyrine dye. The amount of color produced is a function of the concentration of phenolic materials.

3.2 Color response of phenolic materials with 4-amino antipyrine is not the same for all compounds. Because the relative amounts of various phenolic compounds in a given sample are unpredictable, it is not possible to provide a universal standard containing a mixture of phenols. For this reason phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. Because substitution generally reduces response, this value represents the minimum concentration of phenolic compounds present in the sample.

3.3 Most samples require a preliminary distillation to eliminate or reduce to a minimum any interferences that may be present.

3.4 A gas-liquid chromatographic procedure can be used to quantify individual phenolic compounds and in lower concentrations.

4.0 Definitions: The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

4.1 Dissolved Analyte: The concentration of analyte that will pass through a 0.45 um membrane filter assembly prior to sample acidification.

4.2 Phenol: defined as hydroxy derivatives of benzene and its condensed nuclei.

4.3 Instrumental Detection Limit (IDL): The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength.

4.4 Method Detection Limit (MDL): The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

4.5 Linear Dynamic Range (LDR): The concentration range over which the analytical curve remains linear.

4.6 Method of Standard Addition: The standard addition technique involves the use of the unknown and the unknown plus a known amount of standard.

4.7 Laboratory Reagent Blank (LRB): An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, reagents, and acids that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus.

4.8 Calibration Blank: A volume of ASTM type I water acidified with the same acid matrix as in the calibration standards.

4.9 Stock Standard Solution: A concentrated solution containing one analyte purchased from a reputable commercial source. Stock standard solutions are used to prepare calibration solutions and other needed analyte solutions.

4.10 Calibration Standard (CAL): A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to the analyte concentration.

4.11 Instrument Performance Check Solution (IPC): A solution of method analytes, used to evaluate the performance of the instrument system.

4.12 Laboratory Fortified Blank (LFB): An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether method performance is within acceptable control limits.

4.13 Laboratory Fortified Sample Matrix (LFM): An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM evaluated based on the concentrations found.

4.14 Quality Control Sample (QCS): A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB matrix. The QCS is obtained from a source external to the laboratory and is used to check laboratory performance.

4.15 Water sample: For the purpose of this method, a sample taken from one of the following sources: surface, ground, storm runoff, industrial or domestic wastewater.

4.16 Units of weights and measures: g gram, mg milligram, ug microgram, l liter, ml milliliter, ul microliter.

4.17 May: This action, activity, or procedural step is neither required nor prohibited.

4.18 May not: This action, activity, or procedural step is prohibited.

4.19 Must: This action, activity, or procedural step is required.

4.20 Shall: This action, activity, or procedural step is required.

4.21 Should: This action, activity, or procedural step is suggested, but not required.

5.0 Interferences

5.1 Interferences such as phenol-decomposing bacteria, oxidizing and reducing substances, and alkaline pH values can be eliminated by acidification.

5.2 Oxidizing agents such as chlorine and those detected by the liberation of iodine on acidification in the presence of potassium iodide are removed immediately

after sampling by the addition of an excess of ferrous ammonium sulfate. If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

5.3 To eliminate interferences from sulfur compounds such as hydrogen sulfide and sulfur dioxide, acidify the sample to a pH of less than 4 with H_3PO_4 and aerate briefly by stirring and adding $CuSO_4$.

5.4 Oils and tars: Make an alkaline extraction by adjusting to pH 12 to 12.5 with sodium hydroxide pellets. Extract oil and tar from aqueous solution with 50 ml. chloroform. Discard oil or tar containing layer. Remove excess chloroform in aqueous layer by warming on a water bath before proceeding with the distillation step.

6.0 Safety

6. 1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard, and exposure to these compounds should be as low as reasonably achievable through using hoods, gloves and other appropriate personal safety equipment.

6.2 Precautions should also be taken to minimize other potential hazards. Basic good housekeeping and safety practices such as the use of rubber or plastic gloves, lab coat, and safety glasses during handling of samples and cleaning of labware are recommended.

6.3 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved with this method.

6.4 As with all electrical and heated instruments, observe basic safety rules. Do not touch electrical areas and allow surfaces to cool before touching.

7.0 Equipment and Supplies: Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

7.1 Distillation apparatus, all glass consisting of a 1 liter pyrex distilling apparatus

with Graham condenser. See Table 1 for diagram of distillation apparatus.

7.2 pH meter

7.3 Spectrophotometer, for use at 460 or 510 nm. Sequoia-Turner, Model 390.

7.4 Separatory funnels, 500 or 1000 ml. With ground-glass stoppers and TFE stopcocks.

7.5 Filter funnels, Buchner type with fritted disk- 15 ml Corning No. 36060 or equivalent.

7.6 Filter paper: Alternatively use an appropriate 11 cm filter paper for filtering methylene chloride extracts instead of the Buchner-type funnels and anhydrous Na_2SO_4 sodium sulfate.

7.7 Cuvettes or glass tubes with tops.

7.8 Volumetric flasks, 100 ml.

7.9 Erlenmeyer flasks, 250 ml.

8.0 Reagents and Standards

8.1 Concentrated sulfuric acid.

8.2 CAUTION: Prepare under hood, irritating odor. Buffer solution: Dissolve 6.76 g of NH_4Cl (ammonium chloride) in 57.2 ml of NH_4OH (ammonium hydroxide) and dilute to 100 ml with deionized water. Two mL should adjust 100 mL of distillate to pH 10.

8.3 4-Aminoantipyirine solution: Dissolve 1 g of 4AAP in deionized water and dilute to 50 ml.

8.4 Potassium ferricyanide solution: Dissolve 4 g of $K_3Fe(CN)_6$ in deionized water and dilute to 50 ml.

8.5 CAUTION: Toxic, handle with extreme care. Stock phenol solution: Dissolve 1.0 g of phenol in freshly boiled and cooled deionized water and dilute to 1 liter. 1 ml of solution contains 1 mg phenol. 1 gram of liquid phenol equals 1 gram of solid. Prepare stock spiking solution the same as stock standard solution.

8.6 Working solution A: Dilute 5 ml of the stock solution to 500 ml with deionized water. 1 mL= 10 ug phenol.

8.7 Working solution B: Dilute 10 ml of working solution A to 100 ml with deionized water. 1 ml of solution contains 1 µg of phenol.

8.8 Methylene chloride.

8.9 Phosphoric acid solution, 1+9: Dilute 10 ml of 85% H_3PO_4 to 100 ml with deionized water.

8.10 Copper sulfate solution: Dissolve 100 g $CuSO_4*5H_2O$ in deionized water and dilute to 1 liter.

8.11 Sodium hydoxide solution 2.5 N: Dilute 41.7 mL 6N Na OH to 100 mL or dissolve 10 g Na OH pellets in 100 mL deionized water.

9.0 Sample Collection, Preservation and Handling

9.1 The sample is preserved by acidification to a pH of less than 4 with sulfuric acid (about 2 mL of concentrated sulfuric acid per 1 liter)

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9.2 The sample should be kept at 4°C until analyzed.

9.3 The holding time is 28 days.

9.4 If not preserved and stored at 4°C or lower, sample must be analyzed within 4 hours after collection.

10.0 Quality Control

10.1 An LRB (reagent blank) and calibration blank shall be analyzed in each run.

10.2 Spike and duplicate 10% of all samples analyzed with a minimum of one spike and one duplicate per run. Analyze a spiked blank (lab fortified blank) in each run.

10.3 Laboratory fortified sample matrix (LFM or "Spike"): Select a representative water sample of the type of samples being analyzed. It is recommended that this sample be analyzed prior to fortification. The spike amount should be 20% to 50% higher than the analyzed value. Over time, samples from all routine sample sources should be fortified.

10.4 Calculate the percent recovery, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits of 70-130% A recovery calculation is not required if the concentration of the analyte added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{Cs - C}{s} \times 100$$

where, R = percent recovery
Cs = fortified sample concentration
C = sample background concentration
s = concentration equivalent of fortifier added to water sample.

10.5 If analyte recovery falls outside the designated range, and the laboratory performance is shown to be in control, (laboratory performance checks are within the specified range), the recovery problem encountered with the spiked water sample is judged to be matrix related, not system related. The result for each analyte in the unspiked sample must be labeled to inform the data user that the results are suspect due to matrix effects.

10.6 Duplicate data. Duplicates should be studied and for most samples duplicates should fall within 10% of each other. It is suggested that the analysts use their judgement in the evaluation of the duplicate data.

10.7 Laboratory performance check. The values for the laboratory performance checks must fall within the allowable range for each analyte in the performance check used. A check sample should be run with each analysis. Recovered analyte concentrations of this sample must be within acceptable limits or samples must be rerun.

10.8 If the analytical run is suspect due to any of the previous four points, the samples from the section of the analytical run which was determined to be out of control must be rerun following recalibration.

10.9 Samples outside the range of the standards must be diluted before being analyzed.

10.10 Initial Demonstration of performance.

10.10.1 An MDL shall be established for each analyte using reagent water

(blank) fortified at a concentration of two to five times the estimated detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

MDL = (t) x (S)

where: t = value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom is, t = 3.14 for seven replicates.

S = standard deviation of the replicate analysis.

10.10.2 An MDL shall be determined yearly or whenever a significant change in background or instrument response is expected and documented.

10.10.3 Linear calibration ranges: The upper limit of the linear calibration range shall be established for each analyte by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Linear calibration ranges shall be determined yearly or whenever a significant change in instrument response is observed.

10.10.4 Quality control sample (QCS): a quality control sample is analyzed after each calibration and must be within the established limits or the analysis cannot proceed and the source of the problem must be identified and corrected before continuing.

10.11 Assessing Laboratory performance: Reagent and fortified blank

10.11.1 The laboratory must analyze at least one LRB with each set of samples. LRB data are used to assess contamination from the laboratory environment and to characterize spectral ackground form the reagents used in sample preparation. If an analyte value in an LRB exceeds its determined MDL, then laboratory or reagent contamination is suspected. Any determined source of contamination should be eliminated and the samples reanalyzed.

10.11.2 One LFB (spiked blank) must be analyzed with each batch of samples. Calculate accuracy as percent recovery. If recovery of an analyte falls outside control limits of 70-130%, the method is

judged out of control. The source of the problem should be identified and resolved before continuing the analysis.

11.0 Calibration and Standardization

11.1 Follow manufacturer's instructions for calibrating instrument.

11.1.1 Make sure appropriate stray light filter is in position. Turn on instrument and allow to warm up for about one hour.

11.1.2 Place cuvette with blank solution (zero standard) into cuvette holder.

11.1.3 Press and hold ZERO SET button while adjusting ZERO knob until display indicates 0.0. Release ZERO SET button.

11.1.4 Set MODE switch to Absorbance.

11.1.4 Adjust 100%T/0A COARSE knob to approximately 0.000.

11.1.6 Adjust 100%T/0A FINE knob to exactly 0.000.

11.1.5 Replace blanking cuvette with sample cuvette and read Absorbance from digital display. Repeat for all standards and samples. Record all data on printed spreadsheet.

11.2 At least four standards are used, with one near the detection level of 25 ug/L and one higher than the highest value being read, with two in between.

11.3 An Excel spreadsheet is used for all calculations, displaying absoptions, concentrations, and correlation of coefficient.

11.4 The standards are read first and the correlation of coefficient should be at least .99 or better and the calibration check should be within the specified range or samples should be rerun.

12.0 Procedure

12.1 Distillation.

12.1.1 Measure 500 mL of sample into a 500 mL erlenmeyer flask. Adjust pH to about 4 with 2.5 NaOH solution if sample was preserved with H_2SO_4.

12.1.2 Transfer sample to the distillation apparatus. Collect distilled sample in same flask used to measure sample. For solids, weigh out about 10 g of sample and transfer to the distillation apparatus with 500 ml deionized water.

12.1.3 Distill 450 ml of sample, stop the distillation and when boiling ceases add 50 ml of warm distilled water to the flask and resume distillation until 500 ml have been collected.

12.1.4 If the distillate is turbid, either filter through a prewashed membrane filter or distill the sample again.

12.15 Measure 100 ml of each sample into a marked erlenmeyer flask using a 100 ml volumetric flask. Prepare spikes and duplicates as needed, one for every 10 samples. The spike amount used is 300 ug/l. Go to section 12.2.2 for analysis procedure.

12.2 Direct Photometric Method

12.2.1 Using working standard solution A, prepare the following standards as needed in 100-mL volumetric flasks:

Working Solution A (mL)	Concentration (ug/L)
0.0	0.0
0.25	25.0
0.5	50.0
1.0	100.0
2.0	200.0
5.0	500.0
8.0	800.0
10.0	1000.0

12.2.2 CAUTION: Perform the following operations under a hood. To 100 mL of sample distillate in flasks or to an aliquot diluted to 100 mL and/or standards, add 2 mL of buffer solution and mix. The pH of the sample and standards should be 10 ± 0.2.

12.2.3 Add 2.0 mL aminoantipyrine solution and mix.

12.2.4 Add 2.0 mL potassium ferricyanide solution and mix.

12.2.5 After 15 minutes, pour into a matched set of tubes and cap.

12.2.6 Read absorbance at 510 nm and record on printed worksheet. Go to section 13.0 for data analysis.

12.3 Methylene Chloride Extraction (For concentrations below 30 ug/L)

12.3.1 Using working solution B, prepare the following standards. Standards may be prepared by pipetting the required volumes into the separatory funnels and diluting to 500 ml with deionized water.

ml of working solution B	Conc. µg/l
0.0	0.0
3.0	6.0
5.0	10.0
10.0	20.0
20.0	40.0
25.0	50.0

12.3.2 Place 500 ml of distillate or an aliquot diluted to 500 ml in a separatory funnel. The sample should not contain more than 25 mg of phenol.

12.3.3 To the sample and the standards add 10 ml of buffer solution and mix. The pH should be 10 ± 0.2.

12.3.4 Add 3.0 ml of 4-aminoantipyrine solution and mix.

12.3.5 Add 3.0 ml of potassium ferricyanide solution and mix.

12.3.6 After three minutes extract with 25 ml of methylene chloride. Shake the separatory funnel at least 10 times and let the methylene chloride settle. Shake again 10 times and let the methylene chloride settle again. Vent fumes into a hood.

12.3.7 Filter the methylene chloride extract through filter paper. Do not add more methylene chloride. Carryout filtration in a hood. Dispose of methylene chloride in an environmentally acceptable manner.

12.3.8 Read the absorbance of the samples and the standards against the blank at 460 nm. Go to section 13.0 for data analysis.

13.0 Data Analysis, Calculations, and Reporting Results

13.1 Calculations

13.1.1 Prepare a standard curve by plotting the absorbance value of the standards versus the corresponding phenol concentrations. Obtain the concentration value of the samples directly from the standard curve. Or use a computer program see 13.1.2.

13.1.2 An Excel spreadsheet is used for all calculations, displaying absorbances, concentrations, and correlation of coefficient. A blank copy of the spreadsheet should be printed before beginning analysis so log numbers and data can be recorded.

13.2 For µg/g. of sample = $\frac{A \times B}{C}$

Where A: µg/L from standard curve.

B: 500 ml.

C: g. of solid sample used.

14.0 Method Performance

14.1 The MDL was determined according to instructions in 10.10.1 to be 20-30 ug/L.

14.2 The mean and standard deviation for percent recovery of a matrix spike are 103.85% and 8.69, using 12 records. The mean and standard deviation for percent recovery for a lab fortified blank are 103.05% and 9.09 for spike amount using 7 records. The mean and standard deviation for percent recovery of a known sample are 110.33% and 13.53 using 14 records.

15.0 Pollution Prevention

15.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation such as ordering smaller quantities of standards or preparing

reagents in smaller amounts that can be used completely. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

15.2 For information about pollution prevention that may be applicable to laboratories consult *Less is Better: Laboratory Chemical Management for Waste Reduction,* available form the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N. W. , Washington D.C. 20036, 202-872-4477.

16.0 Waste Management

16.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16.2 For further information on waste management consult *The Waste Management Manual for Laboratory Personnel,* available from the American Chemical Society at the address listed in the Section 15.2.

17.0 References

17.1 EPA-600/4-79-020, Methods for Chemical Analysis of Water and Wastes, revised March, 1983, pp. 420.1-1 through 420-4 (EPA Method 420.1).

17.2 Standard Methods For the Examination of Water and Wastewater, 20th Edition, 1998, Method 5530, pp. 5-40 to 5-44.

Table 1. Distillation apparatus